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AGING OF ADULT TROPICAL REEF FISH BY OTOLITHS: A COMPARISON OF THREE METHODS ON DIAGRAMMA PICTUM

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ABSTRACT

Age determinations for Diagramma pictum, (n=102), were conducted using three methods. The two first methods are based on the counts of annuli on respectively whole otolith and otolith cross sections. The third method uses the decrease of otolith daily growth increment (DGI) density with age. The growth curves found with the first two methods are comparable and similar to previous results. However, the analysis of life history traits such as size at first reproduction indicates that these methods are likely to underestimate growth. The use of DGI density yields a faster growth curve. To establish this curve the relationships linking fish size to otolith radius and DGI density to otolith radius are needed. The final growth curve is particularly sensitive to changes in these previous relationships. For this reason all sizes should be well represented in the sample. This method is not recommended for fish with large seasonal growth variations but could produce good DGI measurements for those species which have only a small zone of readible otolith.

INTRODUCTION

Age and growth data are essential for population dynamics studies and for scientific management of stocks and fisheries. This type of information can be obtained by several methods: length frequency data analysis, tag-recapture, seasonal growth increments on hard structures (otolithes, vertebra, scales, dorsal spines, opercular bones, ...), daily growth increments on otoliths and even chemical aging. Generally, the three first methods can be easily applied to temperate fish but in the tropics the aging of adult fish produces a number of problems. A protracted spawning period or multiple spawning periods in the year may render the first method impossible to apply. Sampling problems can complicate the second method and in most cases, annual variations of environmental factors are too slight for clear seasonal growth increments to form. This makes the third method difficult to use. Chemical aging requires a high technology and precise knowledge of the physiology of the species.

Since Panella (1971,1980), a commonly used method consists of reading the daily increments on otoliths. Nevertheless, hyaline and opaque rings, usually called annuli, are sometimes observed in tropical species. In the present study three different aging methods based on otoliths were used on Diagramma pictum (Haemulidae): the count of annuli-like structures on whole otoliths (method 1) and transverse thin sections (method 2) and the estimation of daily growth increments (DGI) on thin sections (method 3).

MATERIAL AND METHODS

The fish used were taken during longline trials or by speargun in various areas of the lagoon of New Caledonia, in 1986 and 1987, at depths ranging from 10 to 15 meters near coral patches. The otoliths were cleaned, measured (Total Length) and one of each pair was embedded in polyester resin and cut into thin sections. Fish size ranged from 9.5 cm to 69.0 cm, with an equal number of fish for each 5 cm class.

First method: whole otoliths

Whole otoliths immersed in anise oil were observed with a binocular microscope (10 x) against a dark background. Under reflected light, opaque and hyaline rings were clearly visible, however, increments were difficult to count on older fish. Reading was performed independantly by two observers. Rings were counted along the posterio-dorsal axis on the external concave side (figure 1). Total radius (Rt) from the nucleus to the outer edge, and the distances (Ri) of each ring from the nucleus were measured. Fish size and Rt were correlated. Since both fish size and otolith radius are dependant variables a Geometric Mean (GM) regression (model II) (Laws and Archie, 1981) was performed. For each average Ri a fish size was estimated from the latter correlation. The Von-Bertalanffy model was applied to this data. As for all other linear and non linear regressions in this paper, the "Statgraphics" package (Statistical Graphics Corporation. Plus Ware * product) was used to make estimates of parameters and of their confidence intervals.

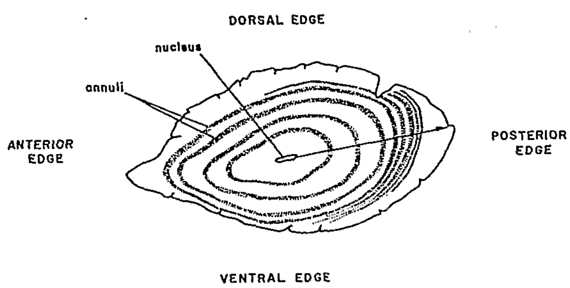

Figure 1. Drawing of a whole left sagitta of Diagramma pictum showing the preferential axis used for counting annuli (arrow).

Second method: annual rings on thin sections

Each embedded otolith was cut through the nucleus. Each half was glued on a glass slide and thin sections (0.25 - 0.5 mm) were made using an Isomet Low Speed Saw. Thin sections were observed using a binocular microscope (40 x) with reflected light and read against a black background. Anise oil enhanced the readibility. All rings were counted, usually in the ventro-proximal edge (figure 2). Ring counts and fish size were correlated using the Von-Bertalanffy model.

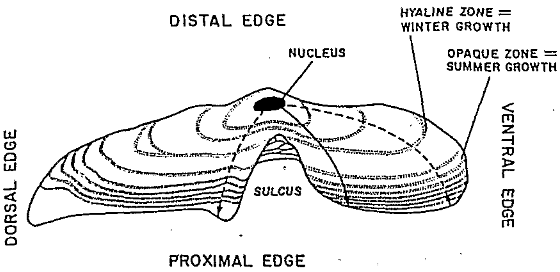

Figure 2. Drawing of an otolith cross section showing areas used for counting rings. Continuous arrow indicates the preferred reading zone.

Third method: daily increments on thin sections.

Thin sections were observed under oil immersion (1250 x). Zones where daily growth increments (DGI) were easily readable and in sufficient numbers, were selected along the dorso-ventral axis, R, which is also measured (figure 3). These zones must however be small enough so that DGI density can be considered constant. On each zone, the number of DGI, the width of the zone and its median distance to the nucleus were measured.

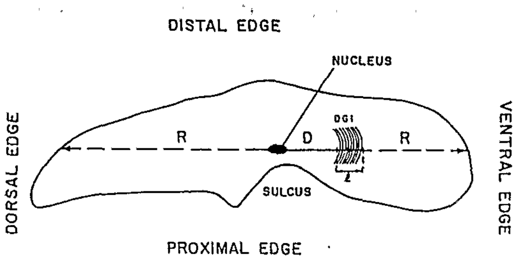

Figure 3. Schematic drawing of an otolith cross section showing measurements made for age determination using DGI. R: otolith radius (dotted line); D: distance between nucleus and counting segment; l: width of counting segment.

Plotting of the final growth curve, linking the age of the fish to its size, calls for the establishement of a number of relationships:

1) relationship between fish size (L) and otolith radius (R). Linear, S-shape (logistic), semi-log and log-log models were fitted using a GM regression. The model giving the lowest residual sum of squares (SSR) was kept.
2) relationship between otolith radius (R) and DGI density (D). Linear, log-log and exponential models were fitted using a GM regression. The model giving the lowest SSR was kept.
3) estimation of the total number of increments (N), i.e. the age of the fish, for a given otolith radius (R). N was calculated according to two methods:
   - integration : for a given Ri, one may write :

$$D = f(R)$$

$$\text{and} \quad N = \int_0^{Ri} f(R)\, dR$$

   - summation (Ralston, 1976,1985) : the radius R was divided into 500 µm intervals. For each of these intervals the average DGI density, di, was calculated. Thus, for a given interval i, the number of DGI is:

$$Ni = di \times 500$$

$$N = \Sigma_{i=1}^{n} Ni$$

Fish size (from step 1) and N for a given otolith radius R were then related using the Von-Bertalanffy model.

RESULTS

First method: whole otoliths

A total of 140 otoliths were examined, of which 14 were rejected as unreadeable by both observers. The relationship between fish size (L) and otolith radius (R) give very similar results for both observers (covariance F test indicates $\alpha >> 0.05$):

observer 1	: L = 1.14 R + 183.33	r = 0.96
observer 2	: L = 1.19 R + 177.17	r = 0.96
combined	: L = 1.16 R + 180.20	r = 0.96

Therefore, it is the equation of the combined observations which was selected. The main advantages of this method are the need of very little equipment (one binocular with a micrometer) and its rapidity (140 otoliths read in two days). However, hyaline rings could be easily separated only up to seven or eigth. Past that number, rings were too close from one another to allow reliable readings. On 20 % of the otoliths the first ring presented ring-like structure in its vinicity. However, these structures were more diffuse than "normal" rings and could be easily separated. The parameters of the Von-Bertalanffy growth model are given in table 1 and the curve is presented on figure 8 (curve 1).

Second method: annuli on thin sections

Thin sections of 61 fish otoliths were examined. For 38 fish two thin sections were available, giving a total of 99 thin sections of which seven were eliminated because of blurred areas. When two readings per fish were available the mean was taken. However, usually both values were similar (35 fish out of 38). Figure 4 indicates the scatterplot of the number of rings versus fish size. The advantage over the previous method was that we were able to differentiate rings near the edge of the otolith (up to 33 rings). However beyond 10 rings (57 cm), growth seemed to be considerably reduced. As for whole otolith readings, the first ring often had ring-like structures in its vinicity.

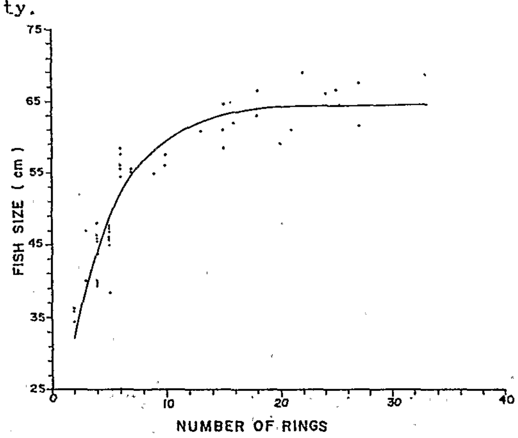

Figure 4. Scatterplot of the number of rings on thin sections versus fish size.

Third method: daily growth increments

A total of 102 otoliths were read, giving 354 density estimates. This method was prefered to total DGI counts for a number of reasons. First because it is much faster and less tedious since counting is confined to a few small segment on each otolith. Furthermore its application is not handicapped by the presence of unreadable areas; a useable measurement can be obtained even if there is only a single readable area on the section. One of the main problems encountered when counting all DGI is to find otoliths presenting good reading along one radius. In older fish, this condition is seldom met, and in Diagramma pictum the number of such otoliths is null past a fish length of 30 cm. However, the use of DGI density presents also a number of problems as will indicate our results.

1) Relationship between fish size (L) and otolith radius (R): the semi-log model gave the best fit to our data (figure 5). However, as indicates this figure, we had no value for radius less than 1200 µm (this corresponds to fish of 7cm in length) and backcalculation of fish size for radius of less than 2000 µm gave abnormally small, even negative sizes. For this reason, a linear model passing through the origin was chosen for radius of less than 2000 µm (L = 0.0137 R, r= 0.84).

2) Relationship between DGI density (D) and otolith radius (R): the exponentiel model gave the best fit for our data (figure 6). However, the variance of D is very high at all radius values. Radke et al. (1985) have shown for Opsanus tau, a temperate species, that DGI density presents marked seasonal variations. DGI density variations could be linked to spawning or to lunar cycles (Panella, 1980) or more accidentally to some kind of stress (food availability, abnormal temperature,...). A possible interpretation is that DGI follow an hypothetical curve as indicates figure 7. If such is the case, the use of time series analysis may help reduce this high variance.

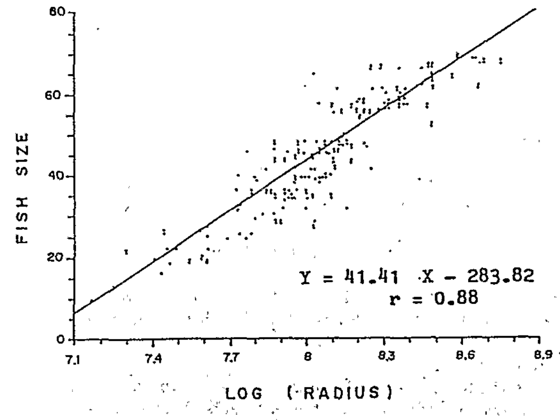

Figure 5. Relationship between fish size and otolith radius.

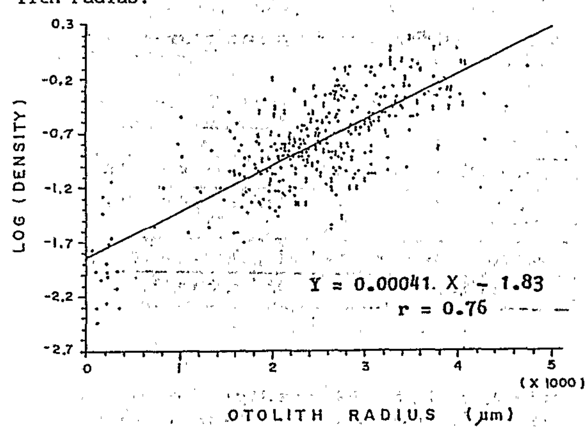

Figure 6. Relationship between DGI density and otolith radius.

DGI readings were homogenously distributed with otolith radius, except for radius values between 200 and 1000 µm for which DGI are usually not possible to count. This corresponds to a fish size between 3.5 and 12 cm. The habitat and ecology of D.pictum at that size are unknown and therefore we can provide no explanation for this phenomenon.

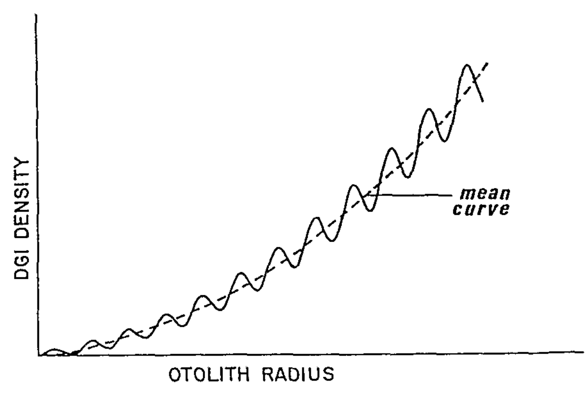

Figure 7. DGI density versus otolith radius hypothetic curve.

3) Estimation of the total number of incre-

ments (N): since the function selected to describe the relationship between D and R is of the form:

$$\text{Log } D = aR + b \implies D = \exp(aR + b)$$

one may write $Ni = \int_0^{Ri} f(R)\, dR$ as

$$Ni = \int_0^{Ri} \exp(aR + b)\, dR$$

or $Ni = \left[1/a \exp(aR + b) \right]_0^{Ri}$

Assuming that N = 0 when R = 0, implies

$$Ni = 1/a \left[\exp(aRi + b) - \exp(b) \right]$$

The growth curve and growth parameters of the Von-Bertalanffy model derived from the values of N and L from this third method are presented on figure 8 and table 1.
The estimates of DGI density and corresponding fish length using Ralston's method are presented in table 2 and the resulting Von-Bertalanffy curve and growth parameters on figure 8 and table 1.

Table 1. Von-Bertalanffy growth parameters obtained by each method.

METHODS	L_∞	k	t_0
WHOLE OTOLITH - 1	58.20 ± 6.78	0.246 ± 0.09	- 0.59 ± 0.48
THIN SECTION - 2 LOUBENS *	64.48 ± 2.38 60.70	0.233 ± 0.06 0.280	- 0.99 ± 1.00 - 0.25
DGI DENSITY-INTEGRATION 3a	69.47 ± 3.40	0.360 ± 0.05	- 0.14 ± 0.11
DGI DENSITY-RALSTON 3b	86.50 ± 12.4	0.210 ± 0.06	- 0.14 ± 0.18

Table 2. Estimates DGI density and corresponding fish length from Ralston's method. N = number of readings; D = DGI density; $ts/\sqrt{n-1}$ = width of the half confidence interval at $\alpha = 0.05$, N-1;
L = calculated fish length for mid class of R.

R CLASSES	0 - 500	500 1000	1000 1500	1500 2000	2000 2500	2500 3000	3000 3500	3500 4000	4000 4500	4500 5000
N	23	3	10	52	93	89	59	20	6	2
Log (D)	0.148	0.320	0.326	0.377	0.436	0.519	0.646	0.747	0.729	0.760
$ts/\sqrt{n-1}$	0.026	0.220	0.084	0.031	0.024	0.034	0.061	0.083	0.209	2.37
L (mm)	34	103	171	240	318	401	471	530	582	628

Table 3. Significant differences in L_∞ and k between all the methods. NS = not significant.

METHODS

L_∞	1	2	3a	3b
1	-	-	-	-
2	NS	-	-	-
3a	*	NS	-	-
3b	*	*	*	-

METHODS

k	1	2	3a	3b
1	-	-	-	-
2	NS	-	-	-
3a	*	*	-	-
3b	NS	NS	*	-

* Significant at $\alpha < 0.05$

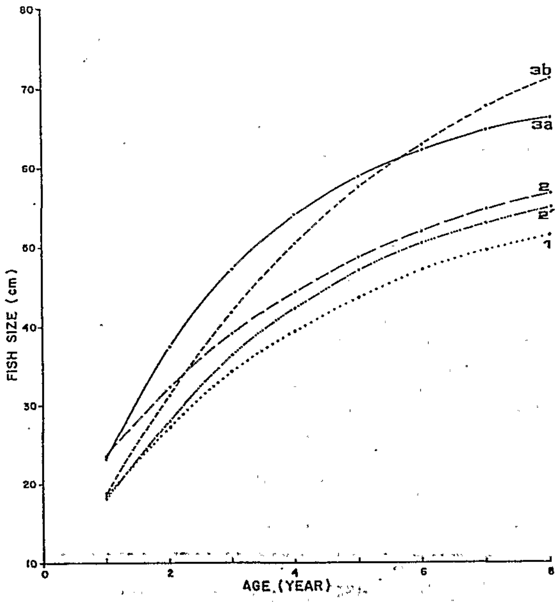

Figure 8. Final growth curves obtained by each method. (1): method 1; (2): method 2; (2'): Loubens's curve; (3a): method 3, DGI density-integration; (3b): method 3, DGI density-Ralston.

Comparison of the three methods

Figure 8 and table 1 regroup the results of all three methods. The findings of Loubens (1978) who used the second method are included for comparison. There is no significant difference of to between any of the methods. Significant differences in L_∞ or k are given by table 3. The first two methods show no difference between one another, whereas method 3a (integration of DGI density) has a larger k than any other method and method 3b has a larger L_∞ than any other method.

DISCUSSION

In the absence of validation, which are conducted at the moment in the Noumea Aquarium, a number of life history traits of Diagramma pictum and a literature survey may explain some of the discrepancies between the results of the various methods. It has been indicated for a large number of fish that the hyaline rings are linked to changes in the environment (temperature, food availability) or physiological changes (reproduction, smolt transformation,...). In temperate waters these changes are seasonal and can thus lead to good correlations between number of hyaline rings and real age. In tropical waters this correlation is questionable because these changes do not necessarily occur on a regular basis. Loubens (1978) has used marginal increment analysis (Campana, 1984; Mason & Manooch, 1985) to check the yearly deposition of these hyaline rings in New Caledonia species. Unfortunatly, he could not validate this hypothesis for D.pictum because of an insufficient sample. Our sample were collected over too short a period to enable us the use of this technique. It is possible to count DGI between two hyaline zones. Practical problems such as localizing the middle of the zone under high magnification, or finding a zone with a continuous reading between two annuli, renders such a verification untractible. The yearly deposit of hyaline rings has been demonstrated in Pakistan for another Haemulid, Pomadasys argenteus, by Brothers & Mathews (1987), but this is a coastal species and the area where this fish live is submitted to the monsoon which is strongly seasonal.

D.pictum matures at 60 cm (Loubens, 1980a). If one assumes that hyaline rings are yearly, this means that this species matures between 10 and 12 years of age and that its growth nearly ends there after. Assuming such an age, frequency size distribution allow to estimate that total mortality rate for mature fish would be of 0.19 which is a very low value considering the high fishing pressure these fish are submitted to. The observation of additional ring-like structures in the vinicity of the first hyaline ring could be related to settlement of this species in coralline zones. Indeed, D.pictum changes drastically of coloration at about 20 cm and starts to form schools around isolated patch reefs. Before this size its main habtat is unknown, occasional specimen being caught by trawlers or seen in shallow sheltered reefs. These life history facts lead us to believe that hyaline rings may not be deposited on a yearly basis, but at a faster rate. An additional argument for non yearly rings is the great proportion of fish larger than L_∞ . This parameter has no real biological meaning, in particular, it is usually inferior to some extent to maximum observed size. However, the examination of frequency size distributions of D.pictum in New Caledonia indicates that 13 % of the fish exceed L_∞ . Similar comparisons of L_∞ (Loubens, 1980b), and frequency size distributions of 12 species in New Caledonia indicate that 8 to 53 % of the fish are larger than L_∞. The magnitude of this discrepancy indicates that the use of hyaline rings is likely to underestimate growth.

The daily periodicity of DGI has been demonstrated for a large number of fish (Campana & Neilson, 1982; Mugiya & Muramatsu, 1982; Victor, 1982; Kingsford & Milicich, 1987; Ralston & Williams, 1988; Ralston & Williams, in prep.; Gjosaeter et al, 1984 and Campana & Neilson, 1985, for reviews), including Haemulids (Brothers & McFarland, 1981; Brothers & Mathews, 1987). However, this applies mainly to young fish, on older fish this is not always verified. In particular, Caillart (pers. communication) indicates for Naso brevirostris and Epinephelus microdon in French Polynesia that such rings may be deposited only every two or three days. By contrast, subdaily increments may also be formed (Taubert & Coble, 1977; Panella, 1980; Marshall & Parker, 1982), which would in turn underestimate growth. Such anomalies occur more frequently past first maturity.

However, the main problem with using DGI density are methodological. First, age estimates require several relationships. Errors for each of these relationships are cumulative. The most sensitive part of this method is the estimation of N (total number of DGI) for a given fish length. This is well illustrated by the significant differences in L and k found using either integration or Ralston's method for estimating N. Integration takes better into account the entire distribution of DGI density, whereas Ralston's method is more robust. However, both methods suffer from autocorrelation, the DGI density at a given age depending of previous life history.

To our opinion, neither method (hyaline rings or DGI density) are satisfactory in their present state. Validation is a must in both cases. However, validation may proove to be difficult since it should be conducted at several sizes and for extended periods (at least several months). In New Caledonia, as in many tropical fisheries, tag-recapture is not conceivable. Aquarium studies, despite their known biases, is our only alternative.

REFERENCES

Brothers, E.B. & McFarland, W.N. 1981. Correlations between otolith microstructure, growth and life history transitions in newly recruited french grunts [Haemulon flavolineatum (Desmarest), Haemulidae]. Rapp. P.v. Reun. Cons. int. Explor. Mer. 1978:369-374.

Brothers, E.B. & Mathews, C.P. 1987. Application of otolith microstructural studies to age determination of some commercial valuable fish of the arabian gulf. Kuwait Bulletin of Marine Science, 9:127-157.

Campana, S.E. & Neilson, J.D. 1982. Daily growth increments in otoliths of starry flounder (Platichthys stellatus) and the influence of some environmental variables in their production. Can. J. Fish. Aquat. Sc. 39: 937-942.

Campana, S.E. & Neilson, J.D. 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sc. 42:1014-1032.

Gjosaeter, J.P., Dayaratine, P., Bergstad, O.A, Gjosaeter, H., Sousa, M.I. & Beck, I.M. 1984. Ageing tropical fish by growth rings in the otoliths. FAO Fisheries circular No 776.

Kingsford, M.J. & Milicich, M.J. 1987. Presettlement phase of Parika scaber (Pisces: Monacanthidae): a temperate reef fish. Mar. Ecol. Prog. Ser. 36:65-79.

Laws, E.A. & Archie, J.W. 1981. Appropriate use of regression analysis in marine biology. Mar. Biol. 65:13-16

Loubens, G. 1978. Biologie de quelques espèces de poissons du lagon néo-calédonien. I- Détermination de l'âge (otolithométrie). cah. ORSTOM, ser. océanogr. 16(3-4):263-283.

Loubens, G. 1980a. Biologie de quelques espèces de poisson du lagon néo-calédonien. II- Sexualité et reproduction. Cahiers de l'Indo-Pacifique 2(1):41-72.

Loubens, G. 1980b. Biologie de quelques espèces de poissons du lagon néo-calédonien. III- croissance. Cahiers de l'indo-Pacifique 2 (2):101-153.

Marshall, S.L. & Parker, S.S. 1982. Pattern identification in the microstructure of sockeye salmon (Oncorhynchus nerka) otoliths. Can. J. Fish. Aquat. Sci. 39:542-547.

Mason, D.L. & Manooch, C.S. 1985. Age and growth of mutton snapper along the east coast of Florida. Fisheries Research 3:93-104.

Mugiya, Y. & Muramatsu, 1982. Time marking methods for scanning electron microscopy in goldfish otoliths. Bull. Jap. Soc. Sci. Fish. 48(9):1225-1232.

Panella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science 173.

Panella, G. 1980. Growth patterns in fish sagittae. In: skeletal growth of aquatic organisms: biological records of environmental change (Topics in geobiology series 1), Rhoads, D.C. & Lutz, R.A. (ed.), Plenum Publ., New york, pp. 519-556.

Radtke, R.L., Fine, M.L. & Bell, J. 1985. Somatic and otolith growth in the oyster toadfish (Opsanus tau L.). J. Exp. Mar. Biol. Ecol. 90:259-275.

Ralston, S. 1976. Age determination of a tropical reef butterflyfish utilizing daily growth rings of otoliths. Fish. Bull. U.S. 74: 990-994.

Ralston, S. 1985. A novel approach to aging tropical fish. National Marine Fisheries Service, N.O.A.A., South west Fisheries Center, Honolulu Laboratory, 7pp.

Ralston, S. & Williams, H.A. 1988. Depth distributions, growth, and mortality of deep slope fishes from the Marianas archipelago. NOAA Technical Memorandum NMFS, 47pp.

Ralston, S. & Williams, H.A. in prep. An improved method of aging exploited tropical fishes using daily growth increments with application to Pristipomoides zonatus in the Marianas archipelago. Southwest Fish. Cent. Honolulu lab., Natl. Mar. Fish. Serv., NOAA, Honolulu.

Taubert, B.D. & Coble, D.W. 1977. Daily rings in otoliths of three species of Lepomis and Tilapia mossanbica. J. Fish. Res. Board Can. 34:332-340.

Victor, B.C. 1982. Daily otolith increments and recruitment in two coral reef wrasses, Thalassoma bifasciatum and Halichoeres bivittatus. Mar. Biol. 71:203-208.